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Two Nutritional Variants of Cultured Jensen Sarcoma Cells.* (24805)

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A previous communication reported that asparagine was essential for growth of the Jensen sarcoma *in vitro*(1). The asparagine requirement could be spared by addition of large amounts of aspartic acid (10-20 mM), but glutamic acid and glutamine were ineffective. During asparagine depletion-repletion experiments, some Jensen cells were observed growing in absence of asparagine. The present report describes development of 2 such nutritional variants of the Jensen sarcoma cultured *in vitro*.

Methods. Tissue culture technics have been described(2). Initial inoculum prepared from intramuscular implants of the tumor in Holtzman rats was 200,000 cells/ml (2 ml total vol. in T-15 flasks). The medium was replenished at end of 48 hours incubation and as often as required thereafter. The substrate used was basal medium 5a(3), and growth was determined at specific time intervals by whole cell count.

Results. When freshly excised Jensen cells were placed in an asparagine-deficient medium most cells became rounded and granular, and total cell population decreased rapidly. At end of 6-7 days of incubation, the number of surviving cells was usually less than 10% of initial inoculum. Some of the remaining cells were viable, since replacing the deficient substrate with the complete medium resulted in cellular proliferation.

When asparagine-depletion experiments were extended beyond an 8-day period, the number of surviving cells continued to decrease and very few viable cells remained. In one such experiment, a different cell type was observed in a flask when the experiment had been in progress for approximately 2 weeks. These cells were elongated and only slightly refractile. Upon continued incubation, no growth was apparent for another week, but after that time there was a definite increase in this particular cell type. As this strain (JA-1) continued to grow, the cells became more refractile and assumed a typical fibroblastic or spindle appearance. There was also a definite tendency to clump or form organoid-like colonies. One month later the JA-1 strain was subcultured and rapid growth was observed. In another series of experiments, a second strain (JA-2) was developed, and the changes which occurred are recorded in Fig. 1.

When JA-1 was implanted into the *rectus femoris* of female Holtzman rats, a tumor developed but at a much slower rate than the Jensen sarcoma. The latency period was approximately 3 weeks as compared to a 4-day latency for the Jensen tumor.

At the end of 5 weeks, strain JA-1 was harvested from the animal and subsequently transplanted into 4 additional rats. Nodules could be palpated after 2 weeks but these regressed after approximately 1 month. Two other attempts to transplant this strain from cells cultured *in vitro* failed, but a 4th try was successful. The period of tumor take was approximately 6 days, but once the tumor had become "established" growth rate was as

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rapid as that observed for the Jensen sarcoma.

Strain JA-2 could also be transplanted *in vivo*. The tumor latency of the first generation was approximately 14 days, but this period became less upon subsequent transplantation. At the end of 8 transplant generations the period of tumor "take" was approximately 6 days but growth rate *in vivo* was comparable to the Jensen sarcoma.

The comparative growth response of freshly excised Jensen sarcoma and strain JA-2 in presence or absence of asparagine can be seen in Fig. 2. In the absence of exogenous as-

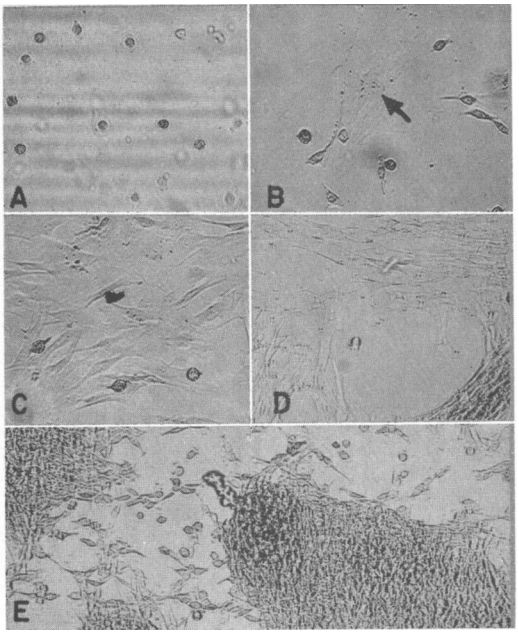

FIG. 1. Development of strain JA-2 of Jensen sarcoma. A, Jensen cells after 7 days in an asparagine-deficient medium; B, first appearance of some variant cells (see arrow) 16 days after inoculation; appearance of the culture, C, 22 days; D, 28 days, and E, 32 days after inoculation. (66×.)

paragine the viable cell population of the Jensen sarcoma markedly decreased. On the other hand, growth of strain JA-2 (in a complete medium or an asparagine-deficient substrate) was comparable to the Jensen sarcoma grown in complete medium 5a. Further, strain JA-2 grew *in vitro* in asparagine-deficient media when removed from animals at various transplant generations (Table I). Similar experiments with JA-1 demonstrated that asparagine was not essential for growth of this strain.

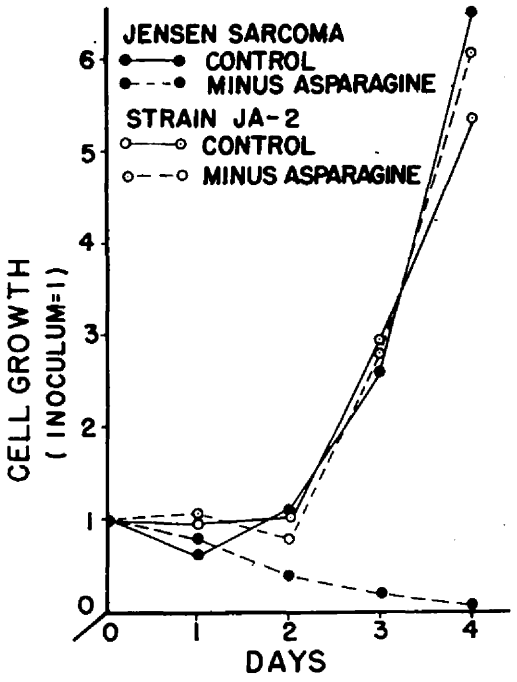

FIG. 2. Comparative growth response of Jensen sarcoma and strain JA-2 to asparagine *in vitro*.

Histological sections of the parent Jensen sarcoma, strains JA-1 and JA-2, were prepared from intramuscular implants. The tissues were fixed in Bouin's solution and stained with hematoxylin and eosin. Examination of the Jensen sarcoma sections revealed the tumor was composed of sheets of neoplastic cells having comparatively abundant pale-staining, finely granular, neutrophilic cytoplasm and indistinct cell membranes. There was moderate variation in cell and nuclear size. Nuclei exhibited single large nucleoli and coarse, deeply stained clumps of chromatin. The sheets of tumor cells were sparsely vascular and presented zones of necrosis. The tumor actively invaded surrounding muscle isolating muscle fibers.

TABLE I. Growth Response of Different Transplant Generations of Freshly Excised JA-2 Cells to Asparagine *In Vitro*.

	Growth in 4-5 days, inoculum = 1					
	In vivo transplant generation					
	1	3	4	5	7	14
Complete medium 5a	4.25	3.71	9.75	1.96	4.46	5.36
Minus asparagine	3.56	3.38	8.95	4.45	4.13	6.05

Strain JA-1 was more sarcomatous in appearance. In general, the cells were smaller and in some areas were elongate exhibiting a tendency to fasciculate. The cells had scanty cytoplasm and the nuclei, although smaller, had the same morphologic characteristics as the Jensen sarcoma. JA-1 appeared to be less invasive and was partially surrounded by a delicate pseudocapsule of fibrous connective tissue. No such zone of connective tissue separated either the Jensen sarcoma or JA-2 from the surrounding muscle.

The most striking difference of JA-2 was its vascularity. Neither of the 2 preceding tissues was highly vascular. The JA-2 presented numerous blood vessels throughout the tumor mass and extensive areas of hemorrhagic necrosis. This necrosis was more marked in strain JA-2 than in the other tumors of similar size. Morphologically the JA-2 more closely resembled JA-1. This variant was highly invasive, actively penetrating the muscle and isolating muscle fibers within the tumor mass. Mitotic activity per high powered field (1000X) appeared to be greater in JA-1 and JA-2 than in the parent Jensen sarcoma. This, however, may have been a function of cell size rather than a true increase in mitotic activity.

Discussion. Nutritional variants of serially propagated cell cultures have been described. Some observers have reported strains which quantitatively differ in the requirement of, or tolerance for, certain substances of unknown molecular structure. For example, Puck and Fisher(4) reported that 2 strains of the HeLa (S_1 and S_3) exhibited high plating efficiency in presence of a feeder system irrespective of high or low serum concentrations. In low amounts of serum but no feeder layer, S_1 exhibited a plating efficiency of practically zero, while plating efficiency of S_3 remained approximately 100%. Similarly, Haff and Swim (5) and Swim and Parker(6) have reported the appearance of nutritional variants when the composition of the medium was altered with respect to serum and embryo extract.

Chang(7) found strains of human cells which could utilize d(+) xylose, d-ribose, or sodium lactate in lieu of glucose. Other reports concerning qualitative nutritional differences between strains have to do with the *meso*-inositol and choline requirements. Chang (8) studied the inositol requirement of different strains of HeLa which had been cultivated in several laboratories and found that the essentiality of this vitamin varied from strain to strain. Further, the essential nature of inositol differed between a wild strain of conjunctival cell and a subculture of this strain grown in a low glucose medium. This difference also occurred between a stable laboratory strain and fresh explants of human amnion cells. In addition, he was able to develop an inositol-less conjunctival cell through deliberate selection in an inositol-deficient medium, but this variant degenerated after approximately 6 months of cultivation. By using similar technics, Swim and Parker(6) isolated 3 strains (U12-79, U12-80, and U12-82) which differed in their respective choline and inositol requirements.

To date, JA-1 and JA-2 strains of the Jensen sarcoma have been grown *in vitro* for 23 and 16 subcultures, respectively, and *in vivo* for 3 and 18 transplant generations, respectively. Both strains have retained the characteristic of growing in the absence of exogenous asparagine under tissue culture conditions. From a morphological standpoint they have not appeared to change and growth characteristics seemed to be consistent.

These findings may have a more important bearing on the problem of mammalian cell nutrition other than development or selection of nutritional variants. For example, if a freshly excised tissue is placed in a medium which does not contain all the essential nutrients for growth, any cells which do proliferate in such a system may represent nutritional variants from the original cell population. This may account for the remarkable similarity of the nutritional requirements of different mammalian cells cultured *in vitro* to date.

Summary. Two nutritional variants (JA-1, JA-2) have been developed from Jensen sarcoma cells cultured *in vitro*. Cells of the parental Jensen sarcoma required asparagine for growth, but those of the variant strains grew readily in the absence of exogenous aspara-

gine. This variation in nutritional requirement has been retained in JA-1 and JA-2 for 23 and 16 subculture generations *in vitro* and in 3 and 18 *in vivo* transplant generations, respectively. Thus, this change was stable and heritable under the conditions described. Some growth and histological characteristics of these variants were described.

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Plasma Ribonuclease Uptake by Rat Kidney Cortex.* (24806)

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Marked increases in serum ribonuclease (RNase) activity follow bilateral nephrectomy, hydronephrosis(1,2) or temporary ligation of the renal pedicles(3); and similar increases are also found in human renal disease (4,5). Further work(6) suggested that serum RNase is inactivated by kidneys, for only minor variations in serum enzyme activity were detected in dogs on which the ureter-venous anastomosis was performed bilaterally. A mechanism of inactivation agreeing with histological and physiological information obtained mainly with administered proteins (*cf.* 7,10), and involving reabsorption of filtered RNase followed by inactivation by the tubular cells was therefore suggested(6,11). It will now be shown that, fitting with above hypothesis, a marked correlation is found between blood serum and kidney cortex RNase activities; further, that enzyme activity increases in the remaining kidney soon after unilateral nephrectomy.

Material and methods. Male Wistar rats, weighing 150-300 g were used. Blood samples of approximately .5 ml were taken from tail tips after warming animals at 37°C for 10 min. Unilateral nephrectomy was performed under ether anesthesia through a lumbar approach. An equal number of left and right kidneys were removed. Sham operated animals were included in several experiments. After removal of kidneys, the capsule was stripped off, and slices less than 1 mm thick were made with razor blade. Half percent homogenates in .85% sodium chloride containing 4% (v/v) n-butanol were prepared with glass homogenizer and Teflon pestle and stored at 2°C. Preliminary experiments showed that the activities obtained with this medium(12) were higher than those of water homogenates. Part of the activity of homogenates, however, was still particle-bound, for it could be sedimented by centrifugation at 5000 g for 15 min. Triplicate samples of 5 μl serum or 50 μl of homogenates were assayed for RNase activity at pH 7.4 as previously described(1,3). Results are expressed in optical density units after subtraction of appropriate blanks. In some experiments "alkaline" phosphatase activity was estimated in 5 μl samples of serum or 20 μl samples of kidney homogenates with disodium phenylphosphate as substrate(13).

Results. Correlation between blood serum and kidney cortex RNase activities. Fig. 1 shows results of 2 experiments in which 31 rats were used. No significant difference

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